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Project No. L6121
Study No. 10

DETERMINATION OF THE CHRONIC MAMMALIAN TOXICOLOGICAL EFFECTS OF RDX

ACUTE DERMAL TOXICITY TEST OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) IN RABBITS

FINAL REPORT

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August 1984

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Chicago, IL 60616

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This study was conducted to evaluate the dermal toxicity of the munitions compounds hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX: CAS Reg. No. 121-82-4) in New Zealand Albino rabbits after a single application of 2 g/kg in 1% carboxymethylcellulose aqueous solution. Toxicological endpoints included body weight measurements, observations of clinical signs and mortality.

Although two males died during the 14-day observation period, gross necropsy and microscopic examination found Tyzzer's Disease as the cause of death. A slight, transient body weight loss was evident for most of the surviving animals, and no other signs of toxicity were observed. To ensure the integrity of the results, the study was repeated only on male rabbits of the same strain but from a different supplier. This repeat study resulted in no mortality.

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Research Toxicologist
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EXECUTIVE SUMMARY

This study was conducted to evaluate the dermal toxicity of the munitions compound hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX; CAS Reg. No. 121-82-4) in New Zealand Albino rabbits after a single application of 2 g/kg in carboxymethylcellulose (1%). Toxicological endpoints included body weight measurements, observations of clinical signs and mortality.

Although two males died during the 14 day observation period, gross necropsy and microscopic examination found Tyzzer's Disease as the cause of death. A slight, transient body weight loss was evident for most of the surviving animals, and no other signs of toxicity were observed. To ensure the integrity of the results, the study was repeated only on male rabbits of the same strain but from a different supplier. This repeat study resulted in no mortality.

The results of acute dermal toxicity of RDX, 2 g/kg in carboxymethylcellulose on single application to rabbit skin show a slight transient body weight loss, no clinical signs of toxicity, and found no mortality in male and female rabbits up to a 14-day observation period.

FOREWORD

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, MD, has been conducting a research program since 1973 for the purpose of developing the scientific data base necessary for recommending water quality criteria for compounds unique to the munitions industry. A water quality criterion (as defined by the amended Clean Water Act, 1977) is a qualitative or quantitative estimate of the concentration of a pollutant in ambient waters that, when not exceeded, will ensure a water quality sufficient to protect a specified water use. The criterion is a scientific entity based solely on data and scientific judgement. It does not reflect considerations of economic or technological feasibility. Currently, a water quality criterion consists of two separate numerical limits, one for the protection of human health and the other for the protection of aquatic organisms. These numbers, when translated by the appropriate regulatory agency, can be the basis of enforceable discharge or effluent limitations in a point source discharge permit issued under the Clean Water Act.

Since a water quality criterion is to protect designated water uses, a diverse, multidisciplinary research program was developed by USAMBRDL that includes "effects" studies on laboratory and domestic animals, wildlife species, aquatic organisms, plants, and economically important crops. In addition, extensive chemical and biological fate and persistence tests are conducted to provide information on the behavior of a pollutant in the aqueous environment. These kinds of data are especially useful for making site-specific translation of criteria into enforceable discharge limits.

This report represents a portion of the mammalian toxicology data base being developed by USAMBRDL on hexahydro-1,3,5-trinitro-1,3,5-triazine.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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ACKNOWLEDGMENT

This report was prepared at IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616, under U.S Department of Army contract No. DAMD17-79-C-9161 (IITRI Project No. L6121) entitled "Determination of the Chronic Mammalian Toxicological Effects of RDX". Mr. Jesse J. Barkley, Jr., Environmental Protection Research Division, USAMBRDL, served as the Contract Officer's technical representative for these programs.

The work reported herein was conducted in the Toxicology and Pharmacology Section of the Life Sciences Division, and represents a portion of the overall effort of the above named research programs. Paul M. Lish, Ph.D., Scientific Advisor, served as Principal Investigator. Barry S. Levine, D.Sc., Senior Toxicologist and E. Marianna Furedi-Machacek, DVM, Research Toxicologist, served consecutively as study director and toxicologist, and were responsible for overall study conduct. Vladislava Rac, DVM, M.S. Senior Veterinary Pathologist, was responsible for the supervision of gross necropsies and microscopic examination of collected tissues. Johnny L. Raymond, experimentalist and Colleen A. Astrauskas, biotechnician, were responsible for collection of test data. Josephine M. Reed M.S., M.M., Supervisor, Quality Assurance, and Julie McPhillip, clerk, were responsible for the quality assurance program. Robert Remaly, B.S., Senior engineer, prepared the test article dosage form. Hugh J. O'Neill, Ph.D., Manager, Analytical Chemistry, and Walter C. Eisenberg, Ph.D., Senior Chemist, were responsible for chemical analyses of the test article.

QUALITY ASSURANCE STATEMENT

Laboratory operations were inspected on August 19 and 20, September 1 and October 15, 1981. The final draft report was audited on May 30, 1984. Inspections and audits were performed by Josephine M. Reed. The study was found to conform to IITRI Life Sciences Quality Assurance criteria. Raw data and specimens generated during the study will be retained in the IITRI Life Sciences Archives.

Josephine M. Reed, MM, MS
Supervisor, Quality Assurance

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I. Introduction

The present study was conducted to evaluate the primary dermal toxicity in rabbits of hexahydro-1,3,5-trinitro -1,3,5-triazine (RDX: CAS Reg. No. 121-82-4) following a single dermal application of 2 g/kg.

During acute and subchronic studies of 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (HMX) it was discovered that this substance had an unexpected and surprisingly high degree of percutaneous toxicity in rabbits (1). Since RDX is closely related chemically to HMX and the RDX composite contains approximately 5-10% of HMX, it was thought prudent to test RDX composite for percutaneous toxicity using the rabbit model.

The study was conducted in two parts. The first part tested five rabbits of each sex. On the basis of the results, five additional males were tested.

The experimental design was based on EPA's Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules Subpart C. Acute and Subchronic Health Effects (2) All laboratory methods and procedures were conducted in accordance with the IITRI Quality Assurance Program designed to conform with FDA Good Laboratory Practices Regulations (3).

II. Material and Methods

A. Test Article

Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS Reg. No. 121-82-4) batch No. HOL 435-37 was stored at the IITRI Kingsbury Ordnance Plant (KOP) Explosive Facility, La Porte, IN at ambient room temperature and humidity, and in the dark. Approximately 30 g reserve samples were taken and stored under similar conditions as the batches.

The purity of the RDX Composite for the aforementioned batch was 91.0 $\pm$ 2.9% as determined by high performance liquid chromatography. The main contaminant was HMX and other impurities were not determined.

B. Dosage Form Preparation

A sufficient quantity of RDX was suspended in 1% carboxymethylcellulose (CMC) (Sigma lot No. 770-0334) to a final concentration of 800 mg/ml (80% w/v).

C. Animals, Quarantine, Housing and Diet

New Zealand Albino rabbits were obtained from two suppliers: 13 males and 13 females were received on August 3, 1981 from Lesser's Rabbitry, Union Grove, WI and eight males were received on September 22, 1981 from the Dutchland Laboratories, Inc., Denver, PA. Random body weights recorded within three days after arrival were 2.15 ± 0.25 kg for the rabbits of both sexes of the first shipment and 1.5 ± 0.1 kg for the males from the second shipment.

The rabbits were housed in air-conditioned rooms at 22-25 degrees C, ambient relative humidity with 12 hr light/12 hr dark cycle. Each rabbit was housed individually in a stainless steel cage which conformed to the upper weight range recommended in the Guide for the Care and Use of Laboratory Animals, DHEW, (NIH) No. 78.23. All animals were transferred to clean cages weekly. Alfalfa pellets (Research Industries, Monee, IL) were placed in the pan below the stainless steel mesh floor of the cage to absorb liquids.

The animals were quarantined for 14 days. During this time one female died. The cause of death was not determined for this animal. The males from the second shipment were quarantined 21 days to achieve the desired weight range for the test.

Purina Rabbit Chow (Ralston Purina Co., St. Louis, MO) and tap water were provided ad libitum from arrival until termination.

D. Test procedure

Following the quarantine period, five animals of each sex and five males for the retest were chosen from the stock using a table of random numbers. All rabbits used in this study appeared to be healthy on the basis of clinical observations (coat, skin, eyes) and body weight. All animals on test received a different test animal number that appeared as a tag on the ear of each rabbit. A card was present on the front of each cage and contained the test animal number, sex, test article, dose (2 g/kg) and study number.

Twenty-four hours prior to testing, fur from the trunk of the animals was clipped so that about a 12 x 20 cm surface area was available for application of the test article. The test area was abraded immediately prior to test article application by making four epidermal abrations with a clean needle through the stratum corneum, but not deep enough to disturb the derma. Following application of the test material on August 19 and October 15, 1981 the test area was covered with gauze, plastic film and a lint-free cotton towel. All wrappings were removed from the animal 24 hours following application and the skin was washed clean of excess test article with distilled water and dried with a gauze pad.

All test animals were observed three times post-treatment on the day of dosing and daily thereafter for 14 days for pharmacologic and/or toxicologic effects. Body weights were recorded for all

animals at the time of test animal selection, on Test Day 1 (immediately prior to dosing) and on Test Days 3, 8 and 15. No post mortem observations were performed except for the two animals that died on test. The two animals that died spontaneously were immediately necropsied under the supervision of the pathologist. Sections of livers, ileum and cecum with lesions were collected, fixed in 10% neutral buffered formalin, histologically processed and stained with Hematoxylin and Eosin. Liver sections were also stained with Giemsa stain which is diagnostic for Bacillus Piliformis, the etiologic factor in Tyzzer's Disease. The final days of observations (Test Day 15) were September 2 and October 29, 1981 for the two parts of the study.

III. Results

A. Mortality and Observations

All five females and three males from the first part of the study and all five males from the retest group of rabbits survived the 14 day observation period. No clinical signs of toxicity were observed for these animals.

Two males from the first group were found dead on Test Day 3 and 9, respectively, following slight body weight loss but without clinical signs of toxicity. Necropsy findings included scattered multiple gray foci, 1 mm diameter, on all liver lobes for both animals and dark red mucosa of the ileum and dark red contents in the cecum for the male found dead on Test Day 9. Based upon gross necropsy lesions and confirmed by microscopic examination by presence of Bacillus Piliformis , Tyzzer's Disease was diagnosed (4) (Appendix).

B. Body Weights

A transient decrease in body weight values was evident for most of the animals on Test Day 3. By the end of the observation period, the surviving animals showed body weight gain. (Tables 1-2).

IV. Conclusion

A single 2 g/kg dermal application of RDX did not appear to be lethal in rabbits. The two male rabbits which died during the observation period demonstrated Tyzzer's Disease upon pathologic examination. This disease process and not RDX was apparently responsible for their deaths.

Study No. L6121-10-1

Table 1

BODY WEIGHTS (KG) OF RABBITS
FOLLOWING A SINGLE DERMAL 2 G/KG DOSE OF
RDX

Test Animal Number	TEST DAY				
	-7	1	3	8	15
	MALES				
13	2.5	2.8	2.8	2.9	3.0
14	2.5	2.3	2.3	—*	—
15	2.6	2.9	2.6	2.2	—**
16	2.8	2.7	2.6	2.9	3.1
17	2.2	2.3	2.2	2.2	2.7
	FEMALES				
18	2.5	2.8	2.6	2.8	3.1
19	2.2	2.4	2.3	2.5	2.5
20	2.4	2.9	2.7	3.0	3.1
21	2.5	2.8	2.7	2.9	3.0
22	2.3	2.5	2.4	2.6	2.6

* Test Animal No. 14 died during Test Day 3

** Test Animal No. 15 died during Test Day 9

Table 2

BODY WEIGHTS (KG) OF RABBITS
FOLLOWING A SINGLE DERMAL 2 G/KG DOSE OF
RDX

Test Animal Number	TEST DAY				
	-3	1	3	8	15
	MALES				
01	2.2	2.2	2.0	1.7	2.2
02	2.2	2.2	2.0	2.2	2.4
03	2.1	2.1	2.1	2.3	2.5
04	2.2	2.4	2.2	2.0	2.4
05	1.8	1.9	1.8	2.0	2.2

V. References

1. Cuthbert, J.A., D'Arcy-Burt, K.J., Carr, S.M.A. HMX, Acute Toxicity Tests in Laboratory Animals. Inveresk Research International Ltd., Final Report, DAMD-17-80-C-0053, 1985

2. Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules Subpart C. Acute and Subchronic Health Effects 40 CFR, Part 722. 44067, 1979

3. Good Laboratory Practice Regulations. Fed. Reg. 21 CFR Part 38. 60013-60020, 1978

4. Allen, A. M., Ganaway, J. R., Moore, T. D. and Kinard, R. F. Tyzzer's disease syndrome in laboratory rabbits. Am. J. Pathol. 46: 859-882, 1965

APPENDIX

MICROSCOPIC OBSERVATIONS

Study Number L6121.10
Dose (mg/l) _____
Test Animal Number 14
Accession Number 81-7175

Microscopic Observations:

Liver - Moderate, focal disseminated hepatocellular necrosis, cellular debris with degenerating polymorphonuclear leukocytes

Gimsa Stain - *Bacillus piliformis* was observed in several hepatocytes at the perithery of necrotic foci

Cecum - Diffuse severe necrosis with hemorrhage of mucosal epithelium. Moderate bacterial growth on denuded mucosal surface. Moderate diffuse submucosal edema

Ileum - Diffuse, mild mucosal necrosis. Slight diffuse accumulation of polymorphonuclear leukocytes

Kidney - unilateral - no deviation from normal morphology

Typist B. Letica
Verified: [signature]

Vladislav S. Foe 10.16.81
Pathologist - Date

Study Number L6121.10
Dose (mg/l) _____
Test Animal Number 15
Accession Number 81-7176

Microscopic Observations:

Liver - Multifocal hepatocellular necrosis, multifocal accumulation of cellular debris with degenerating polymorphonuclear leukocytes

Giemsa Stain - At the periphery of necrotic foci few hepatocytes contain *Bacillus piliformis*

Cecum - Diffuse, severe necrosis of mucosal epithelium with bacterial colonies. Diffuse, moderate hemorrhage in submucosa. Diffuse, moderate edema with scattered inflammatory cells in submucosa

Ileum - Diffuse, moderate necrosis of mucosal epithelium. Mild, diffuse accumulation of inflammatory cells in mucosa and submucosa

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